

CLAIMS

What is claimed is:

1. A method for the accelerated production of transgenic animals homozygous for a selected trait comprising:
transfecting a non-human mammalian cell-line with a given transgene construct containing at least one DNA encoding a desired gene;
selecting a cell line(s) in which the desired gene has been inserted into the genome of that cell or cell-line;
performing a first nuclear transfer procedure to generate a first transgenic animal heterozygous for the desired gene;
characterizing the genetic composition of said first heterozygous transgenic animal;
selecting cells homozygous for the desired transgene through the use of a selective agent;
characterizing surviving cells using known molecular biology methods; and picking surviving cells or cell colonies cells for use in a second round of nuclear transfer or embryo transfer; and producing a second transgenic animal homozygous for a desired transgene.
2. The method of claim 1, wherein said first transgenic animal is biopsied so as to characterize the genome of said first transgenic animal.
3. The method of claim 2, wherein the cells or cell line biopsied from said first transgenic animal is expanded through cell culture techniques.
4. The method of claim 1, wherein said surviving cell are characterized by one of several known molecular biology methods including without limitation FISH, Southern Blot, PCR.
5. The method of claim 1, wherein homozygous transgenic animals are more quickly developed for xenotransplantation purposes or developed with humanized Ig loci.

6. The method of claim 1, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from an ungulate.
7. The method of either claims 1 or 6, wherein said donor cell or donor cell nucleus is from an ungulate selected from the group consisting of bovine, ovine, porcine, equine, caprine and buffalo.
8. The method of claim 1, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is from an adult non-human mammalian somatic cell.
9. The method of claim 1, wherein said non-human mammal is a rodent.
10. The method of claim 1, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is a non-quiescent somatic cell or a nucleus isolated from said non-quiescent somatic cell.
11. The method of either claims 1 or 6, wherein the fetus develops into an offspring.
12. The resultant offspring of the methods of claim 1.
13. The resultant offspring of claim 1 further comprising wherein the offspring created as a result of said nuclear transfer procedure is homozygous for more than one desired gene.
14. The method of claim 1 further comprising using a second selective agent.
15. The method of claim 14 such that the transgenic homozygous cell lines selected can proceed through a second or more multiple rounds selection to generate a cell line homozygous for more than one desired gene.
16. The method of claim 1, wherein cytocholasin-B is used in the cloning protocol.

17. The method of claim 1, wherein cytocholasin-B is not used in the cloning protocol.
18. The method of claim 1, wherein said donor differentiated mammalian cell to be used as a source of donor nuclei or donor cell nucleus is a non-quiescent somatic cell or a nucleus isolated from said non-quiescent somatic cell.
19. The resultant offspring of the methods of claims 1 or 18.
20. The method of claim 1, wherein the techniques used to generate a homozygous cell line are used to develop a functional organ for transplantation.
21. The method of claim 20, wherein said cultured inner cell mass cells are used in organogenesis.
22. The method of claim 1 wherein the desired gene codes for a biopharmaceutical protein product.
23. The method of claim 22 wherein said biopharmaceutical protein product is a compound selected from the group consisting of: antithrombin III, lactoferrin, urokinase, PF4, alpha-fetoprotein, alpha-1-antitrypsin, C-1 esterase inhibitor, decorin, interferon, ferritin, transferrin conjugates with biologically active peptides or fragments thereof, human serum albumin, prolactin, CFTR, blood Factor X, blood Factor VIII, as well as monoclonal antibodies.
24. The method of claim 1 wherein the DNA construct containing the desired gene is actuated by at least one beta casein promoter.
25. The resultant milk derived from the offspring of the methods of claim 1 or 24.